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## ABSTRACT

During the period October 15-24, 1999, an attempt was made to collect biopsy samples, via the use of an 18 foot outboard motor boat and cross bows, of skin and blubber from coastal bottlenose dolphins, *Tursiops truncatus*, at two sites: Ocean City, Maryland (Figure 1) and Chincoteague Island, Virginia (Figure 1). Following two days of searching at the Ocean City, Maryland site we detected no animals, and the site was abandoned. The field collection team moved 35 miles south to Chincoteague Island, Virginia where coastal bottlenose dolphin were located. Following a brief survey of the Chincoteague Inlet area, the team determined that sufficient dolphins (n = 200 - 250) were in the area to begin biopsy operations. The team worked with this group of dolphins during the following 3.5 days. Nine biopsy samples were collected. These biopsy samples will support ongoing genetic and stable isotope analyses for determining stock structure and foraging ecology of coastal bottlenose dolphin in the Northwest Atlantic Ocean.

## INTRODUCTION

The coastal migratory stock of bottlenose dolphins, *Tursiops truncatus*, was designated as depleted under the Marine Mammal Protection Act of 1972 (MMPA) as a result of an epizootic in the late 1980s. At that time no information existed on stock structure of coastal bottlenose dolphin along the Southeast coast of the United States. As a result of the listing, it became necessary to gain a better understanding of how many stocks exist in the Southeast, the extent of their ranges, and their relationship to bottlenose dolphin found offshore (Mead and Potter, 1995). One aspect of the ongoing stock identification project is to use genetic information to examine stock structure, and to use biopsy techniques to collect skin samples for the genetic analyses. It was determined at a 1997 stock identification workshop (Hohn 1997), that samples collected from bottlenose dolphins inhabiting coastal waters north of Chesapeake Bay in the summer were essential for this study. Only in this area can coastal bottlenose dolphins be unequivocally classified as belonging to the "coastal migratory stock" which was designated and listed as depleted under the MMPA. The biopsy field sampling project describe here was initiated to obtain the necessary skin samples from animals north of the Chesapeake Bay for genetic analysis( Hoelzel et al.,1998), and blubber samples for stable isotope analysis (Walker and Macko, 1999; Walker et al., 1999).

The purpose of this paper is to document cross bow biopsy techniques used to collect skin and blubber samples from coastal bottlenose dolphin. Observations on behavioral responses of bottlenose dolphins to vessel approach are also provided.

## METHODS

The goal of the field sampling trip was to obtain 30 to 40 biopsy samples from coastal bottlenose dolphins inhabiting the area north of the Chesapeake Bay. The samples were collected for use in ongoing genetic (P. Rosel, NMFS, Charleston, SC) and stable isotope (S. Wetmore, University of Massachusetts) analyses aimed at determining stock structure and foraging ecology of coastal bottlenose dolphins in the waters of the Southeastern United States.

One week prior to departure to the study site, a letter was drafted and sent to local law enforcement agencies, including the Coast Guard, the local police department and the marine patrol. This letter explained our reason for, and method of, collection, how long we would be in the area, and included a copy of the Northeast Fisheries Science Center Marine Mammal Permit #917.

Two members of the biopsy team arrived in Ocean City, Maryland on October 14, 1999. Using an 5.33 meter rigid-hull inflatable boat (RHIB) powered with a 60 horsepower outboard motor, the team surveyed the inland waters from northern Chincoteague Bay to Isle of Wright Bay north of Ocean City, Maryland on October 16th and 17th (Figure 1). Due to the paucity of dolphins at the Ocean City site on October 18, 1999, the team moved to Chincoteague Island, Virginia, 35 nautical miles south of Ocean City, Maryland. They were joined by two more members on October 20th, adding a 2nd marksman and a recorder/sample processor. Survey operations began on the morning of October 19 and were conducted in and around the Chincoteague Inlet area, both inside the bay and along the outer beaches (Figure 1). Several groups of dolphins were located within the first hour of searching and biopsy procedures were begun. When a group of dolphins was sighted, the boat was maneuvered to within 7-9 meters of their location.

Two different techniques were used in approaching dolphins. During the aggressive approach protocol, the boat operator constantly pursued the animal changing speed and course as necessary. In the passive approach, the operator did not change boat speed once the initial approach was made.

Biopsies were collected using 150lb pull cross bows, manufactured by Barnett International, fitted with 25mm cutting heads mounted on carbon fiber arrow shafts with molded flotation around the shaft so that the arrows could be retrieved (designed by Ceta-Dart, F. Larsen, Copenhagen, Denmark). Date, time, location, shooter(s), number of shots, hits and samples collected were recorded for each biopsy attempt, as described by Mesnick et al. (1999). Location, latitude and longitude were recorded using a global positioning system. Surface sea water temperature data were supplied by the United States Coast Guard, Chincoteague, Virginia.

When a biopsy sample was collected, it was removed from the dart head using ethanol cleaned forceps, wrapped in aluminum foil, put in a plastic bag, and placed on ice. In the laboratory, a portion of blubber from each biopsy was removed using a fresh razor blade, and then wrapped in foil and frozen at -20°C. The remaining tissue was placed in a labeled vial of 20% DMSO/saturated sodium chloride and stored at room temperature ( Barrett-Lennard et al., 1996).

Biopsy sampling trips in the Chincoteague Inlet area were conducted during 19th - 23<sup>rd</sup> October, 1999.

## RESULTS

Although local fishing boat captains and the Coast Guard had indicated that dolphins were present in the Ocean City, Maryland area in early October, no dolphins were seen during two days (October 15-16, 1999) of extensive surveying of the area. At the Chincoteague Inlet site, dolphins were relatively abundant between October 19-21, but their numbers declined between October 21 and 23. By October 23<sup>rd</sup>, no animals were seen in the Chincoteague Inlet area, nor during surveys north and south of the inlet along the outer beaches. An additional day, October 24, was spent searching only to again yield negative results. During the period from October 19-23 the surface sea water temperature declined from 17.8 degrees C to 15.6 degrees C.

When dolphins were seen, the boat was maneuvered to within 8-11 meters. However, it was noted that when animals were in shallow water (1-2 meters deep), it was easier to control their movements, perhaps because their options to avoid approach were limited. When the boat neared a group of animals, they would most often surface and roll once, and then dive for two to four minutes. Water visibility was poor, and as a result there was no warning for the marksmen that an animal was about to surface. A total of 76 shots were taken, resulting in 11 hits and 9 biopsy samples (Table 1). The total number of hits vs shots was 14.5%. There were two instances in which an animal was hit, but no sample was obtained. In one case, the biopsy bolt did not release and the animal swam off with it. In the second case, the biopsy hit the dorsal fin but no sample was retained.

On October 19 a single marksman shooting from the bow of the RHIB took 17 shots and attained six hits and collected five biopsies (Table 1). The percentage of hits vs shots was 35.3%. There were approximately five hours of biopsy effort on this day. During this sampling the aggressive approach was used.

On October 21, the passive approach was used during six hours of biopsy efforts with two marksmen at the bow of the RHIB. A total of 43 shots were taken, four hits were made and three biopsies were collected (Table 1). The percentage of shots vs. hits was 9.3%.

On October 22, during approximately three hours of sampling effort the aggressive approach was used with two marksmen. One marksman was stationed at the bow of the RHIB and a second marksman/helmsman was stationed at the steering console. The marksman/helmsman was tasked with covering the aft 180 degree sector. Eight shots were taken, one hit was made and one biopsy collected. The percentage of shots vs. hits was 12.5%.

## DISCUSSION

Although only nine samples were collected during this pilot study, the biopsy team concluded that, at the right time of year, sufficient samples could be collected from bottlenose dolphins in this area. It was noted that it would be useful to take dorsal fin photos (Karczmarski and Cockcroft, 1998) of animals biopsied whenever possible; data sheets need to be modified to include such information. The Chincoteague Inlet area may be an appropriate area to conduct live captures because of its hard, sandy bottom topography and shallow waters. An ongoing cooperative biopsy program between the Southeast Fisheries Science Center and the Northeast Fisheries Science Center is important in obtaining samples for determining the stock structure of bottlenose dolphin for the entire east coast.

It should be noted that weather, wind, and rain were factors in the low collection rate.

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TABLE 1. BOTTLENOSE DOLPHIN DATA RECORD 10/19/1999 - 10/22/1999

DATE	TIME	LAT	LONG	GROUP SIZE	SHOT#	HIT	BIOPSY?	COMMENTS
10/19	0919	37°52'.0	75°24'.0	1	1			DCP
10/19	0930	37°52'.0	75°24'.0	1	1			DCP
10/19	0944	37°52'.0	75°24'.0	5	1	1	1	DCP
10/19	1015	37°52'.0	75°24'.0	3/1calf	1			DCP
10/19	1115	37°52'.0	75°52'.9	1	1			DCP
10/19	1210	37°52'.9	75°24'.4	6/2calf	1	1	1	DCP
10/19	1215	37°52'.9	75°24'.4	5/1calf	1	1	1	DCP
10/19	1217	37°52'.9	75°24'.4	5/1calf	1			DCP
10/19	1300	37°52'.1	75°25'.2	5	1			JRN
10/19	1302	37°52'.3	75°29'.9	3	2			JRN
10/19	1305	37°52'.3	75°24'.9	1	1			DCP
10/19	1411	37°52'.2	75°24'.9	6	1			DCP
10/19	1412	37°52'.3	75°24'.9	6	2	1		JRN/DCP
10/19	1420	37°52'.3	75°24'.9	2	1	1	1	DCP
10/19	1424	37°52'.3	75°24'.9	4	1	1	1	DCP
<b>Sub-Total</b>					<b>17</b>	<b>6</b>	<b>5</b>	
10/20	0920	37°53'.0	75°24'.8	2	1			DCP
10/20	0923	37°53'.0	75°24'.8	1	1			DCP
10/20	0927	37°53'.0	75°24'.7	1	1			DCP
10/20	0931	37°52'.0	75°24'.7	1	1			DCP
10/20	0935	37°52'.0	75°24'.7	1	1			DCP
10/20	0943	37°52'.0	75°24'.8	1	2			DCP
10/20	0946	37°52'.0	75°24'.8	2	1			DCP
<b>Sub-Total</b>					<b>8</b>	<b>0</b>	<b>0</b>	
10/21	0852	37°53'.7	75°24'.4	3	1	1	1	DCP

DATE	TIME	LAT	LONG	GROUP SIZE	SHOT#	HIT	BIOPSY?	COMMENTS
10/21	0908	37°53'.7	75°24'.4	3	1			CWP
10/21	0924	37°53'.7	75°24'.4	2	1			DCP
10/21	0932	37°53'.1	75°24'.7	1	1			DCP
10/21	0939	37°53'.1	75°24'.6	1	1			CWP
10/21	0945	37°53'.1	75°24'.6	1	1			DCP
10/21	0947	37°53'.1	75°24'.5	1	2	1	1	DCP
10/21	0958	37°53'.1	75°24'.5	1	1			DCP
10/21	1008	37°52'.3	75°24'.9	1	1			DCP
10/21	1010	37°52'.3	75°24'.9	1	2			DCP/CWP
10/21	1013	37°51'.9	75°25'.3	1	1			CWP
10/21	1150	37°53'.6	75°24'.4	1	1	1		CWP
10/21	1200	37°53'.6	75°24'.4	2	1			DCP
10/21	1208	37°53'.6	75°24'.4	1	1			DCP
10/21	1220	37°52'.3	75°25'.2	2	2			DCP/CWP
10/21	1222	37°52'.3	75°25'.2	1	1			DCP
10/21	1328	37°52'.6	75°25'.1	1	1			DCP
10/21	1334	37°52'.6	75°24'.9	1	1			DCP
10/21	1340	37°52'.4	75°24'.4	4	2			DCP/CWP
10/21	1343	37°52'.4	75°24'.4	3	1			CWP
10/21	1347	37°52'.2	75°24'.1	4	1			CWP
10/21	1405	37°52'.4	75°24'.5	5	2			DCP/CWP
10/21	1411	37°52'.8	75°24'.6	2	2			CWP
10/21	1428	37°52'.8	75°24'.6	4	1	1	1	DCP
10/21	1442	37°52'.5	75°25'.0	3	1			DCP
10/21	1445	37°52'.3	75°25'.2	5	1			CWP
10/21	1446	37°52'.3	75°25'.2	3	1			DCP
10/21	1453	37°52'.7	75°25'.1	1	2			DCP/CWP
10/21	1457	37°52'.4	75°25'.1	4	3			DCP/CWP
10/21	1503	37°52'.4	75°25'.1	5	1			DCP
10/21	1505	37°51'.9	75°25'.3	3	1			CWP

DATE	TIME	LAT	LONG	GROUP SIZE	SHOT#	HIT	BIOPSY?	COMMENTS
10/21	1510	37°51'.9	75°25'.3	4	3			DCP/CWP
<b>Sub-Total</b>					<b>43</b>	<b>4</b>	<b>3</b>	
10/22	1036	37°52'.9	75°24'.8	2	1			CWP
10/22	1117	37°49'.1	75°29'.9	3	2			CWP
10/22	1130	37°48'.9	75°30'.1	3	1			CWP
10/22	1306	37°51'.6	75°25'.6	1	1	1	1	CWP
10/22	1318	37°51'.6	75°25'.6	3	1			CWP
10/22	1320	37°51'.6	75°25'.6	2	2			CWP/JRN, bad weather
<b>Sub-Total</b>					<b>8</b>	<b>1</b>	<b>1</b>	
<b>Total</b>					<b>76</b>	<b>11</b>	<b>9</b>	

DCP - David Potter  
CWP - Charles Potter  
JRN - John Nicolas

Figure 1. The two study sites occupied during October 15 - 24, 1999 while conducting biopsy sampling of bottlenose dolphins. Ocean City Maryland to the North and Chincoteague, Virginia to the South.

